

AmpliSens® HSV I, II-FRT PCR kit



For Professional Use Only

Instruction Manual

KEY TO SYMBOLS USED

| | | | |
|--|---------------------|--|-----------------------------------|
| | Catalogue number | | Caution |
| | Batch code | | Contains sufficient for <n> tests |
| | Research Use Only | | Use-by Date |
| | Version | | Consult instructions for use |
| | Temperature limit | | Keep away from sunlight |
| | Manufacturer | | Negative control of amplification |
| | Date of manufacture | | Negative control of extraction |
| | Internal control | | Positive control of amplification |

1. INTENDED USE

AmpliSens® HSV I, II-FRT PCR kit is not a medical device. PCR kit is an *in vitro* nucleic acid amplification test for qualitative detection of *Simplexvirus humanalpha 1, 2* (HSV types I and II) DNA in the biological material (urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge), discharge from the rectal mucosa, conjunctival discharge, discharge from erosive-ulcerative elements of the skin and mucosa, swab from the mucous membrane of the oropharynx, whole venous blood, cerebrospinal fluid, prostate gland secretion, urine (first portion)) using real-time hybridization-fluorescence detection of amplified products. The material for PCR is DNA samples extracted from test material.

Indications and contra-indications for use of the reagent kit

The reagent kit is used for the analysis of biological material taken from persons with suspected herpes virus infections, without distinction of form and presence of disease manifestation.

There are no contra-indications with the exception of cases when the material cannot be taken for medical reasons.

NOTE: For research use only. Not for diagnostic procedures

2. PRINCIPLE OF PCR DETECTION

Principle of testing is based on the DNA extraction from the samples of test material with the exogenous internal control sample (Internal Control-FL (IC)) and simultaneous amplification of DNA fragments of the detected microorganism (*Simplexvirus humanalpha 1, 2*) and DNA of the internal control with hybridization-fluorescence detection. Exogenous internal control (Internal Control-FL (IC)) allows to control all PCR-analysis stages of each individual sample and to identify possible reaction inhibition.

Amplification of DNA fragment is performed with the use of specific primers and Taq-polymerase enzyme. In the real-time PCR, the amplified product is detected with the use of fluorescent dyes. These dyes are linked to oligonucleotide probes, which bind specifically to the amplified product during thermocycling. The real-time monitoring of fluorescence intensities during the real-time PCR allows the detection of accumulating product without re-opening the reaction tubes after the PCR run.

AmpliSens® HSV I, II-FRT PCR kit uses "hot-start", which greatly reduces the frequency of nonspecifically primed reactions. "Hot-start" is guaranteed by using chemically modified polymerase (TaqF). The chemically modified polymerase (TaqF) is activated by heating at 95 °C for 15 min.

The PCR kit contains the system for prevention of contamination by amplicons using the enzyme uracil-DNA-glycosylase (UDG) and dUTP.

The results of amplification are registered in the following fluorescence channels:

Table 1

| Channel for fluorophore | FAM | JOE |
|-------------------------|---|-----------------------------------|
| DNA-target | <i>Simplexvirus humanalpha 1, 2</i> (HSV types I, II) DNA | Internal Control (IC) |
| Target gene | glycoprotein <i>gpB</i> gene | Artificially synthesized sequence |

3. CONTENT

AmpliSens® HSV I, II-FRT PCR kit is produced in 2 forms:

variant FRT-100 F, R-V8-F(RG,iQ)-CE;

variant FRT-100 F in bulk¹, R-V8-F(RG,iQ)-CE-B.

Variant FRT-100 F includes:

| Reagent | Description | Volume, ml | Quantity |
|-------------------------------|---|------------|----------|
| PCR-mix-1-FL HSVI, II | clear liquid from colorless to light lilac colour | 1.2 | 1 tube |
| PCR-mix-2-FRT | colorless clear liquid | 0.3 | 2 tubes |
| Polymerase (TaqF) | colorless clear liquid | 0.03 | 2 tubes |
| Positive Control complex (C+) | colorless clear liquid | 0.2 | 1 tube |
| DNA-buffer | colorless clear liquid | 0.5 | 1 tube |
| Negative Control (C-)* | colorless clear liquid | 1.2 | 1 tube |
| Internal Control-FL (IC)** | colorless clear liquid | 1.1 | 1 tube |

* must be used in the extraction procedure as Negative Control of Extraction.

** add 10 µl of Internal Control-FL (IC) during the DNA extraction procedure directly to the sample/lysis mixture (see the DNA-sorb-AM, DNA-sorb-B, MAGNO-sorb or AmpliSens®MAGNO-sorb-URO protocols).

Variant FRT-100 F is intended for 110 reactions (including controls).

4. ADDITIONAL REQUIREMENTS

For sampling and pretreatment

- Transport medium.
- 0.9 % sodium chloride solution (sterile saline solution).
- Endocervical brush.
- Swabs for collecting biological material, single use, sterile.
- Vacuum tubes or disposable system for collecting venous blood for *in vitro* laboratory tests.
- Sterile double-sided tubular needles for vacuum tubes for venous blood collection for *in vitro* laboratory tests.
- Plastic container (50-60 ml) for storage and transportation of biological samples, single use, sterile.
- Vacuum tube for urine with stabilizer.
- Disposable tightly closed polypropylene 1.5-ml tubes.
- Screwing caps for tubes.
- Disposable tips for variable volume pipettes up to 200 and 1000 µl.
- Tube racks.
- PCR box.
- Desktop centrifuge up to 12,000 g (suitable for Eppendorf tubes).
- Vortex mixer.
- Vacuum aspirator with flask for removing supernatant.
- Pipettes (adjustable).
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

For DNA extraction and amplification

- DNA extraction kit or Automated station for DNA extraction based on magnetic beads with MAGNO-sorb and AmpliSens®MAGNO-sorb-URO nucleic acid extraction kit
- Set of consumables for used automated station according to the manufacturer's recommendations.
- Sterile RNase-free pipette tips with aerosol filters (up to 100, 200 and 1000 µl).
- Tube racks.
- PCR box.
- Vortex mixer.
- Pipettes (adjustable).
- Real-time instruments (for example, Rotor-Gene Q (QIAGEN, Germany), Rotor-Gene 6000 (Corbett Research, Australia), CFX 96 (Bio-Rad, USA)).
- Disposable polypropylene tubes for PCR kit variant FRT-100 F:
 - a) screwed or tightly closed 1.5-ml tubes for reaction mixture preparation.
 - b) thin-walled 0.2-ml PCR tubes with optical transparent domed or flat caps or strips of eight 0.2-ml tubes with optical transparent caps if a plate-type instrument is used;
 - c) thin-walled 0.2-ml PCR tubes with flat caps or strips of four 0.1-ml Rotor-Gene PCR tubes if a rotor-type instrument is used.
- Refrigerator for 2–8 °C.
- Deep-freezer at the temperature from minus 24 to minus 16 °C.
- Disposable powder-free gloves and a laboratory coat.
- Reservoir for used tips.

5. GENERAL PRECAUTIONS

The user should always pay attention to the following:

- Use sterile pipette tips with aerosol filters and use a new tip for every procedure.
- Store all extracted positive material (specimens, controls and amplicons) away from all other reagents and add it to the reaction mix in a distantly separated facility.
- Thaw all components thoroughly at room temperature before starting an assay.
- When thawed, mix the components and centrifuge briefly.
- Use disposable protective gloves and laboratory cloths, and protect eyes while samples and reagents handling. Thoroughly wash hands afterwards.
- Do not eat, drink, smoke, apply cosmetics, or handle contact lenses in laboratory work areas.
- Do not use the PCR kit if the internal packaging was damaged or its appearance was changed.

¹ In bulk form contains unlabeled tubes. Tubes with identical reagent are packed in one bag with label.

- Do not use the PCR kit if the transportation and storage conditions according to the Instruction Manual were not observed.
- Do not use a kit after its expiration date.
- Dispose of all specimens and unused reagents in accordance with local regulations.
- Samples should be considered potentially infectious and handled in biological cabinet in compliance with appropriate biosafety practices.
- Clean and disinfect all samples or reagents spills using a disinfectant, such as 0.5 % sodium hypochlorite or another suitable disinfectant.
- Avoid inhalation of vapors, samples and reagents contact with the skin, eyes, and mucous membranes. Harmful if swallowed. If these solutions come into contact, rinse the injured area immediately with water and seek medical advice if necessary.
- While observing the conditions of transportation, operation and storage, there are no risks of explosion and ignition.
- Safety Data Sheets (SDS) are available on request.
- The PCR kit is intended for single use for PCR analysis of specified number of samples (see the section "Content").
- The PCR kit is ready for use in accordance with the Instruction Manual. Use the PCR kit strictly for intended purpose.
- Use of this product should be limited to personnel trained in DNA amplification techniques.
- Workflow in the laboratory must be one-directional, beginning in the Extraction Area and moving to the Amplification and Detection Area. Do not return samples, equipment and reagents in the area where the previous step was performed.



Some components of this kit contain sodium azide as a preservative. Do not use metal tubing for reagent transfer.

6. SAMPLING AND HANDLING

PCR kit is intended for analysis of the DNA extracted with the use of DNA extraction kits from the biological material:

- urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge),
- discharge from the rectal mucosa,
- discharge from the mucous membrane of the oropharynx,
- erosive-ulcerative elements of the skin and mucosa,
- prostate gland secretion,
- conjunctival discharge,
- urine (first portion),
- whole venous blood,
- cerebrospinal liquid.

NOTE: When using EDEM reagents kit for extraction of DNA by express method, test material (except urine) is collected only in tubes with **Transport Medium TM-EDEM** included in this reagent kit. EDEM reagent kit is used for initial screening of patients and is not intended for monitoring after treatment.

NOTE: If **Transport Medium with Mucolytic Agent** is used, the color of the liquid may change at an acidic pH.

Sampling

Urogenital mucous discharge

Vaginal mucous discharge

Collect the material from the posterolateral vaginal vault. Use the working part of the probe to rotate along the surface of the lateral walls of the vagina, collecting the discharge as much as possible. Minimal presence of impurities in the form of mucus and blood is acceptable.

Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

It is not allowed to use scissors to cut the working part of the probe!

Scraping from the mucous membrane of the cervical canal

The cervical canal should be accessed using a disposable or reusable sterile gynecological speculum. Before obtaining the material, remove mucus and vaginal discharge from the surface of the cervix with a sterile gauze swab (minimal presence of impurities in the form of cervical mucus and blood is acceptable). The material should be taken using an endocervical brush (cytocerical brush) or a combined gynaecological probe (it is allowed to use in the examination of pregnant women, young nulliparous women).

Methods for taking scrapings of epithelial cells:

- A cervical epithelial scraping (endocervix), taken with one cytobrush, and/or a cervical surface epithelial scraping (ectocervix) taken with a second cytobrush should be placed in a tube with transport medium.
- A scraping of the cervical epithelium (endocervix and ectocervix) taken with a combined gynaecological probe should be placed in a tube with transport medium.

Break off the working part of the cytobrush/probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

It is not allowed to use scissors to cut the working part of the probe!

Urethral mucous discharge

Female: before taking a urethral scraping, treat the external opening of the urethra with a swab moistened with a sterile 0.9% sodium chloride solution to remove discharge from the vaginal discharge. Insert the working part of the probe into the urethra to a depth of 1–2 cm, with several rotary movements to collect the discharge. The presence of impurities such as mucus and blood is acceptable.

Male: before taking a urethral scraping, treat the glans penis in the area of the external opening of the urethra with a swab moistened with a sterile 0.9% sodium chloride solution. Massage the urethra. Any discharge flowing free from the urethra should be removed with a dry swab. Insert the working part of the probe into the urethra to a depth of 1–2 cm, and collect the discharge with several rotational movements. The presence of impurities such as mucus and blood is acceptable.

Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

Discharge from the mucous membrane of the oropharynx

Use the working part of the swab probe to move with rotational movements along the surface of the tonsils, palatine glands and the posterior wall of the oropharynx. Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

It is not allowed to use scissors to cut the working part of the probe!

Conjunctival discharge

The procedure should be performed under local anesthesia, (for example, using 2 drops of Dicaïne (0.3% solution)). After applying the anesthesia, the lower eyelid should be pulled back and the working part of the tampon probe should be moved along the conjunctiva, capturing the inner and outer corners of the eye, 4–5 times. Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

It is not allowed to use scissors to cut the working part of the probe!

Samples of urogenital mucous discharge, discharge from the mucous membrane of the oropharynx, conjunctival discharge in a transport medium can be stored before the PCR analysis:

When using Transport Medium with Mucolytic Agent:

- at the temperature from 18 to 25 °C - for 28 days;
- at the temperature from 2 to 8 °C - for 3 months;
- at the temperature from minus 20 °C and below - for a long time.

When using Transport Medium TM-EDEM from the set of reagents for DNA extraction using the EDEM express method:

- at the temperature from 18 to 25 °C - for 2 days;
 - at the temperature from 2 to 8 °C - for 14 days;
 - at the temperature from minus 20 °C and below - for a long time.
- Only one freeze-thawing cycle is required.

Discharge from the rectal mucosa

Thoroughly clean the area around the anus with soap and water. Insert the probe into the anus to a depth of 3–4 cm. The working part should be rotated along the surface of the sidewalls of the anal canal and the rectum. The discharge should be collected as completely as possible. Impurities in the form of mucus, blood, pus and feces can be present. Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

It is not allowed to use scissors to cut the working part of the probe!

Samples of discharge from the rectal mucosa in the Transport medium with mucolytic agent can be stored and transported before the PCR analysis:

- at the temperature from 18 to 25 °C - for 28 days;
- at the temperature from 2 to 8 °C - for 3 months;
- at the temperature from minus 20 °C and below - for a long time.

Erosive-ulcerative elements of the skin and mucosa

Use the working part of the swab probe to move with rotational movements along the surface of the erosive-ulcerative elements of the skin. Transfer the probe to a test tube with 0.5 ml of transport medium. Break off the working part of the probe containing the test material and leave it in a test tube with the transport medium. Close the test tube tightly with the cap, ensuring that there is no gap or wrinkling of the inner part of the cap. If it is impossible to break, the working part of the probe should be immersed in the transport medium and pressed against the inner side of the tube. Rotate for 5–10 s, after which remove the probe and close the test tube tightly.

It is not allowed to use scissors to cut the working part of the probe!

When using Transport Medium with Mucolytic Agent:

- at the temperature from 18 to 25 °C - for 28 days;
- at the temperature from 2 to 8 °C - for 3 months;
- at the temperature from minus 20 °C and below - for a long time.

When using Transport Medium TM-EDEM from the set of reagents for DNA extraction using the EDEM express method:

- at the temperature from 18 to 25 °C - for 2 days;
 - at the temperature from 2 to 8 °C - for 14 days;
 - at the temperature from minus 20 °C and below - for a long time.
- Only one freeze-thawing cycle is required.

Urine (first portion)

Collect the first portion of morning urine without using the toilet of the external genitalia or at least 2 hours after the last urination in a volume of 15–30 ml in a container, tightly close the cap.

For men when urinating, it is necessary to completely pull back the skin fold to release the external opening of the urethra.

When using a vacuum tube for urine with a stabilizer for storage and transportation: mix the urine sample by inverting it in the original container, insert the cap of the vacuum tube into the sampling device (needle holder). Press down until the needle of the device/holder pierces the cap of the test tube (do not remove the cap from the test tube!), fill the test tube and then remove it from the device/holder. Turn the tube over 6–8 times to thoroughly mix the urine with the stabilizer.

Native urine samples can be stored and transported before the PCR analysis:

- at the temperature from 18 to 25 °C - for 1–2 hours;
- at the temperature from 2 to 8 °C - for 1 day;
- at the temperature from minus 20 °C and below - for 7 days;
- at the temperature not higher than minus 68 °C - for a long time.

Urine samples in vacuum tubes can be stored and transported before the PCR analysis:

- at the temperature from 18 to 25 °C - for 8 hours;
 - at the temperature from 2 to 8 °C - for 2 days;
 - at the temperature from minus 24 to minus 16 °C - for 3 months;
 - at the temperature not higher than minus 68 °C - for a long time.
- Only one freeze-thawing cycle is required.

Prostate gland secretion

Before obtaining prostate gland secretion, treat the glans penis with a tampon moistened with a 0.9% sodium chloride solution. The doctor should do taking the prostate secretion after preliminary massage of the prostate gland. After completing the prostate massage, collect its secretion in a volume of at least 0.5–1.0 ml into a test tube or container and tightly close the cap.

If it is impossible to obtain the secretion, immediately after massage of the prostate gland, the first portion of urine (which contains the secretion of the prostate gland) in a volume of 15–25 ml (see rules for collecting urine) should be collected.

Samples of prostate gland secretion can be stored and transported before the PCR analysis:

- at the temperature from 18 to 25 °C - for 6 hours;
 - at the temperature from 2 to 8 °C - for 1 day;
 - at the temperature from minus 20 °C and below - for 7 days;
 - at the temperature not higher than minus 68 °C - for a long time.
- Only one freeze-thawing cycle is required.

Whole venous blood

It is recommended to take whole venous blood after overnight fasting or 3 hours after a meal from the ulnar vein with a disposable needle into a test tube (special vacuum system) with a 6% EDTA solution (K₂EDTA or K₃EDTA) or with sodium citrate as an anticoagulant. Immediately after sampling, gently turn the closed tube with blood upside down several times so that the blood in the tube with the anticoagulant is thoroughly mixed (otherwise,

the blood will clot and DNA extraction will become impossible). Heparin cannot be used as an anticoagulant! Whole venous blood samples can be stored and transported before the PCR analysis:

- at the temperature from 18 to 25 °C – for 2 hours;
- at the temperature from 2 to 8 °C – within 3 days from the moment of taking biological material;

Freezing whole venous blood samples is unacceptable!

Cerebrospinal fluid

Puncture the lumbar, suboccipital area or cerebral ventricles with puncture needles and collect at least 1 ml cerebrospinal fluid by aspiration method into a tube or container. Close the tube or container tightly with a cap. Cerebrospinal fluid samples can be stored and transported before the PCR-analysis:

- at the temperature from 2 to 8 °C - for 1 day;
- at the temperature from minus 24 to minus 16 °C - for 3 months;
- at the temperature not higher than minus 68 °C - for a long time.

Only one freeze-thawing cycle is required.

Pretreatment

Pretreatment for the samples of urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge), discharge from the rectal mucosa, discharge from erosive-ulcerative elements of the skin and mucosa, conjunctival discharge, swabs from the mucous membrane of the oropharynx, urine (first portion), prostate gland secretion, whole venous blood and cerebrospinal fluid is not required.

Urine samples are to be pretreated.

Urine pretreatment

Pretreatment of urine samples for subsequent DNA extraction with DNA-sorb-AM and AmpliSens® MAGNO-sorb-URO reagent kits

Mix the urine sample in the original container. Transfer 1 ml of material into a 1.5-ml tube using a filter tip. Centrifuge for 5 minutes at 12.000 rpm. Remove the supernatant using a non-filter tip and vacuum aspirator, leaving 100 µl of supernatant and pellet. Use the obtained sample for DNA extraction.

Urine sediment samples can be stored before the PCR analysis:

- at the temperature from 2 to 8 °C – for 1 day;
- at the temperature from minus 20 °C and below – for 7 days;
- at the temperature not higher than minus 68 °C – for a long time.

Pretreatment of urine samples for subsequent DNA extraction with the EDEM reagent kit

Mix the urine sample in the original container. Add 1 ml of urine into a test tube with Transport Medium TM-EDEM (0.5 ml), using a separate tip with a filter for each sample. Centrifuge for 5 minutes at 12.000 rpm. Without affecting the sediment, remove the supernatant into the trap flask using a vacuum aspirator, using a separate tip without a filter for each sample. Add 0.5 ml of Transport Medium TM-EDEM to each test tube with urine pellet. Close the tubes tightly, mix the contents thoroughly with vortex to resuspend the sediment, and precipitate drops from the tube walls and the inside of the cap by brief centrifugation. Use the obtained sample for DNA extraction.

Urine sediment samples can be stored in the Transport Medium TM-EDEM:

- at the temperature from 18 to 25 °C – for 2 days;
- at the temperature from 2 to 8 °C – for 14 days;
- at the temperature from minus 20 °C and below – for a long time.

Interfering substances and limitations of using test material samples

In order to control the DNA extraction efficiency and possible reaction inhibition the Internal Control (Internal Control-FL (IC)) is used in the PCR kit. The Internal Control is added in each biological sample at the extraction stage. The presence of internal control signal after the amplification testifies the effectiveness of nucleic acid extraction and the absence of PCR inhibitors.

Samples of biological material are unsuitable for research if the conditions of collection, storage and transportation are violated

Potential interfering substances

Endogenous and exogenous substances that may be present in the biological material used for the study were selected to assess potential interference (see Table 2).

Model samples of biological material (urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge), discharge from the rectal mucosa, discharge from erosive-ulcerative elements of the skin and mucosa, conjunctival discharge, swabs from the mucous membrane of the oropharynx, urine (first portion), prostate gland secretion and cerebrospinal fluid) without adding and with the addition of potentially interfering substances were tested. The concentration of each potentially interfering substance is listed in Table 2.

Quality control sample (QCS) with HSV I DNA at concentration of 1x10⁴ GE/ml was added to the model samples.

Table 2

| Type of tested material | Type of potential interferent | Potential interferent | Tested concentration in a sample | Nucleic acid extraction kit | Interference presence |
|---|-------------------------------|----------------------------|----------------------------------|--|-----------------------|
| Urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge) | Endogenous substances | Mucin | 150 µg/ml | DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO, EDEM | Not detected |
| Swab of the oropharynx mucous | Exogenous substances | Miramistin | 0.2 % | | |
| Discharge of erosive and ulcerative elements of the skin and mucous membranes | Exogenous substances | Iodine (potassium iodide) | 0.5 % | | |
| Conjunctival discharge | Exogenous substances | Dicain | 0.03 % | DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO, EDEM | Not detected |
| Urine (first portion) | Endogenous substances | Albumin | 500 mg/l | DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO, EDEM | Not detected |
| | Exogenous substances | Azithromycin | 1 mg/ml | DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO, EDEM | Not detected |
| Discharge from the rectal mucosa | Endogenous substances | Feces | 5 % | DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO | Not detected |
| | Exogenous substances | Lubricant "Context Strong" | 15 % | DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO | Not detected |

| Type of tested material | Type of potential interferent | Potential interferent | Tested concentration in a sample | Nucleic acid extraction kit | Interference presence |
|--------------------------|-------------------------------|---|----------------------------------|--|-----------------------|
| Prostate gland secretion | Endogenous substances | Fructose | 10 mg/ml | DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO | Not detected |
| | Exogenous substances | Ibuprofen | 300 µg/ml | | Not detected |
| Whole venous blood | Endogenous substances | Hemoglobin | 250 g/l | DNA-sorb-B, MAGNO-sorb | Not detected |
| | Exogenous substances | Potassium EDTA | 2.0 µg/ml | | |
| Cerebrospinal fluid | Endogenous substances | Glucose | 10 mmol/l | DNA-sorb-B, MAGNO-sorb | Not detected |
| | Exogenous substances | Iodine, an alcohol solution for external use 5% | up to 0.04 % volume | | |

7. WORKING CONDITIONS

AmpliSens® HSV I, II-FRT PCR kit should be used at the temperature from 20 to 28 °C and relative humidity from 15 to 75 %.

8. PROTOCOL

8.1. DNA extraction

It is recommended to use the following nucleic acid extraction kits for different types of test material:

| DNA-sorb-AM, AmpliSens® MAGNO-sorb-URO | EDEM | DNA-sorb-B, MAGNO-sorb |
|---|--|---|
| <ul style="list-style-type: none"> - urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge); - discharge from the rectal mucosa; - swab from the mucous membrane of the oropharynx; - conjunctival discharge; - discharge from erosive-ulcerative elements of the skin; - urine (first portion); - prostate gland secretion | <ul style="list-style-type: none"> - urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge); - swab from the mucous membrane of the oropharynx; - conjunctival discharge; - urine (first portion) | <ul style="list-style-type: none"> - whole venous blood; - cerebrospinal liquid |

NOTE: Extract the DNA according to the manufacturer's protocol.

MAGNO-sorb and AmpliSens® MAGNO-sorb-URO nucleic acid extraction kits can be used in combination with "open type" automatic nucleic acid extraction stations. The DNA extraction is carried out in accordance with the Instruction manual.

The DNA extraction of each test sample is carried out in the presence of **Internal Control-FL (IC)**. Each group of extractable samples must include one repeat of the Negative Control of Extraction (C-) which goes through all stages of the PCR study, starting with the extraction stage. C- allows you to control the possible contamination of test samples.

The volumes of reagents and samples when the DNA is extracted by DNA-sorb-AM nucleic acid extraction kit:

Add 10 µl of Internal Control-FL (IC) to each tube with samples.

The volume of the test sample is 100 µl.

Add 100 µl of **Negative Control (C-)** to the tube labeled C- (Negative Control of Extraction).

The volume of elution is 100 µl.

The volumes of reagents and samples when the DNA is extracted by DNA-sorb-B nucleic acid extraction kit:

Add 10 µl of Internal Control-FL (IC) to each tube with samples.

The volume of the test sample is 100 µl.

Add 100 µl of **Negative Control (C-)** to the tube labeled C- (Negative Control of Extraction).

The volume of elution is 50 µl.

The volumes of reagents and samples when the DNA is extracted by AmpliSens® MAGNO-sorb-URO nucleic acid extraction kit:

Add 10 µl of Internal Control-FL (IC) to each tube with samples.

The volume of the test sample is 100 µl.

Add 100 µl of **Negative Control (C-)** to the tube labeled C- (Negative Control of Extraction).

The volume of elution is 100 µl.

The volumes of reagents and samples when the DNA is extracted by MAGNO-sorb nucleic acid extraction kit:

Add 10 µl of Internal Control-FL (IC) to each tube with samples.

The volume of the test sample is 200 µl.

Add 200 µl of **Negative Control (C-)** to the tube labeled C- (Negative Control of Extraction).

The volume of elution is 100 µl.

The volumes of reagents and samples when the DNA is extracted by EDEM reagents kit:

NOTE: Internal Control-FL (IC) is contained in **IC-diluent** reagent. Complementary addition of Internal Control-FL (IC) to the test samples and controls is not required.

The volume of the test sample is 100 µl of Transport Medium TM-EDEM, containing test sample.

Add 100 µl of **Transport Medium TM-EDEM** to the tube labeled C- (Negative Control of Extraction).

8.2. Preparing PCR

8.2.1 Preparing tubes for PCR

The type of tubes depends on the PCR instrument used for analysis.

Use disposable filter tips for adding reagents, DNA and control samples into tubes.

The total reaction volume is 25 µl, the volume of DNA sample is 10 µl.

1. Calculate the required quantity of each reagent for reaction mixture preparation. For one reaction:

- 10 µl of PCR-mix-1-FL HSV I, II,
- 5 µl of PCR-mix-2-FRT,
- 0.5 µl of Polymerase (TaqF).

Prepare the reaction mixture for the total number of test and control samples plus some extra reaction (see numbers of control samples in item 7).

NOTE: Reaction mixture components should be mixed just before PCR analysis.

2. Vortex the tubes with **PCR-mix-1-FL HSV I, II, PCR-mix-2-FRT**, and **polymerase (TaqF)** to mix the content and sediment drops.

3. Prepare the reaction mixture in a separate test tube. Mix the required amount of **PCR-**

- mix-1-FL *HSV I, II*, PCR-mix-2-FRT, and polymerase (TaqF), and sediment the drops by vortex
- Take the required number of the tubes/strips for amplification of the DNA obtained from test and control samples.
 - Transfer 15 µl of the prepared mixture to each tube. Discard the unused reaction mixture.
 - Add 10 µl of DNA samples obtained at the extraction stage.

NOTE: Avoid transferring the sorbent together with the DNA samples extracted with the reagent kit for extraction on silica gel or magnetic separation.

| | |
|---|---|
| 7. Carry out the control amplification reactions: | |
| NCA | – Add 10 µl of DNA-buffer to the tube labeled NCA (Negative Control of Amplification). |
| C+ | – Add 10 µl of Positive Control complex to the tube labeled C+ (Positive Control of Amplification). |
| C– | – Add 10 µl of the sample extracted from the Negative Control (C–) reagent to the tube labeled C– (Negative Control of Extraction). |

8.2.2. Amplification

- Create a temperature profile on your instrument as follows:

Table 3

| Step | Rotor-type Instruments ² | | | Plate-type Instruments ³ | | |
|------|-------------------------------------|------------------------|--------|-------------------------------------|------------------------|--------|
| | Temperature, °C | Time | Cycles | Temperature, °C | Time | Cycles |
| 1 | 95 | 15 min | 1 | 95 | 15 min | 1 |
| 2 | 95 | 20 s | 5 | 95 | 20 s | 5 |
| | 60 | 20 s | | 60 | 20 s | |
| | 72 | 15 s | | 72 | 15 s | |
| 3 | 95 | 20 s | 40 | 95 | 20 s | 40 |
| | 60 | Fluorescence acquiring | | 60 | Fluorescence acquiring | |
| | 72 | 15 s | | 72 | 15 s | |

Table 4

| Step | Plate-type Instruments ⁴ | | |
|------|-------------------------------------|------------------------|--------|
| | Temperature, °C | Time | Cycles |
| 1 | 95 | 15 min | 1 |
| 2 | 95 | 5 s | 5 |
| | 60 | 20 s | |
| | 72 | 15 s | |
| 3 | 95 | 5 s | 40 |
| | 60 | Fluorescence acquiring | |
| | 72 | 15 s | |

Fluorescent signal is detected in the channels for the FAM and JOE fluorophores (other channels are enabled if several tests are simultaneously carried out in a single run).

- Adjust the fluorescence channel sensitivity according to the *Important Product Information Bulletin*.
- Insert tubes into the reaction module of the device.
- Run the amplification program with fluorescence detection.
- Analyze results after the amplification program is completed.

9. DATA ANALYSIS

Analysis of results is performed by the software of the real-time PCR instrument used by measuring fluorescence signal accumulation in two channels:

Table 5

| Channel for the fluorophore | FAM | JOE |
|-----------------------------|--|------------------------------|
| Amplification product | <i>Simplexvirus humanalpha 1,2 (HSV types I, II) DNA</i> | Internal Control-FL (IC) DNA |

Results are interpreted by the crossing (or not-crossing) the fluorescence curve with the threshold line set at the specific level that corresponds to the presence (or absence) of a Ct value of the DNA sample in the corresponding column of the results grid.

The result of the analysis is considered reliable only if the results obtained for Positive and Negative Controls of amplification as well as for the Negative Control of extraction are correct (see Table 6).

Table 6

| Control | Stage for control | Ct value in the channel for fluorophore | |
|---------|-------------------|---|-----------------|
| | | FAM | JOE |
| C- | DNA extraction | Absent | <boundary value |
| NCA | PCR | Absent | Absent |
| C+ | PCR | <boundary value | <boundary value |

Interpretation of some test samples is not possible if the results for the controls deviate from the results specified above (see 10. Troubleshooting).

Principle of interpretation is the following:

Table 7

| Results interpretation | | |
|---|----------------------------|--|
| Ct value in the channel for the fluorophore | | Result |
| FAM | JOE | |
| absent | < boundary value | <i>Simplexvirus humanalpha 1,2 DNA is NOT detected</i> |
| determined | determined or absent | <i>Simplexvirus humanalpha 1,2 DNA is detected</i> |
| absent | absent or > boundary value | Invalid* result |

* In case of invalid result, the PCR analysis should be repeated for the corresponding test

² For example, Rotor-Gene 6000 (Corbett Research, Australia), Rotor-Gene Q (QIAGEN, Germany).

³ For example, CFX96 (Bio-Rad Laboratories, Inc.), USA).

sample starting from the DNA extraction stage.

NOTE: Boundary Ct values are specified in the *Important Product Information Bulletin* enclosed to the PCR kit.

10. TROUBLESHOOTING

Results of analysis are not taken into account in the following cases:

- The Ct value for the Positive Control of PCR (C+) is absent or exceeds the boundary value in the channel for the FAM and/ or JOE fluorophores. It is impossible to interpret the results for all samples. It is necessary to repeat the PCR analysis, starting from the amplification stage.
- For the Negative Control of Extraction (C–):
 - the Ct value is determined in the channel for the FAM fluorophore. The contamination of laboratory with amplification fragments or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *HSV I, II* DNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for these samples starting from the DNA extraction stage.
 - the Ct value is determined in the channel for the JOE fluorophore is greater than the boundary value or absent. This means that Negative Control of Extraction (C–) has not performed the extraction control function. The PCR analysis should be repeated for all samples starting from the DNA extraction stage.
- For the Negative Control of PCR (NCA):
 - the Ct value is determined in the channel for the FAM fluorophore. The contamination of laboratory with amplification fragments or cross-contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *HSV I, II* DNA is detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for these samples, starting from the amplification stage.
 - the Ct value is determined in the channel for the JOE fluorophore. The contamination of laboratory with amplification fragments or contamination of reagents / test samples is probable at any stage of PCR analysis. It is impossible to interpret the results for samples in which *HSV I, II* DNA is not detected. Measures for detecting and elimination of contamination source must be taken. The PCR analysis should be repeated for these samples, starting from the amplification stage.
- If the Ct value is determined for the test sample, whereas the area of typical exponential growth of fluorescence is absent (the graphic looks like approximate straight line). It is necessary to check the correctness of selected threshold line level or parameters of base line calculation. If the result has been obtained with the correct level of threshold line (base line), the amplification and detection should be repeated for this sample.

11. TRANSPORTATION

AmpliSens® HSV I, II-FRT PCR kit should be transported at 2–8 °C for no longer than 10 days.

12. STABILITY AND STORAGE

All components of the **AmpliSens® HSV I, II-FRT** PCR kit are to be stored at 2–8 °C when not in use (except for polymerase (TaqF) and PCR-mix-2-FRT). All components of the **AmpliSens® HSV I, II-FRT** PCR kit are stable until the expiry date on the label. PCR kit **variant FRT-100 F** can be stored without unpacking at 2 to 8 °C for 3 months from the date of manufacture before opening. Once opened, PCR kit **variant FRT-100 F** should be unpacked in accordance with the storage temperatures for each component. After the opening of the primary container, the reagents must be used within the shelf life of the reagent kit, unless otherwise stated. The shelf life of the reagent kit is stated on its label.

NOTE: Polymerase (TaqF) and PCR-mix-2-FRT are to be stored at the temperature from minus 24 to minus 16 °C when not in use.

NOTE: PCR-mix-1-FL *HSV I, II* is to be kept away from light.

13. SPECIFICATIONS

13.1. Analytical sensitivity

| Test material | Transport medium | Nucleic acid extraction kit | Analytical sensitivity (limit of detection), GE/m |
|---|---------------------------------|---|---|
| Urogenital mucous discharge (vaginal mucous discharge, scraping from the mucous membrane of the cervical canal and urethral mucous discharge) | Transport Medium with Mucolytic | DNA-sorb-AM AmpliSens®MAGNO-sorb-URO | 1x10 ³ |
| | Transport Medium TM-EDEM | EDEM | |
| Swab from the mucous membrane of the oropharynx | Transport Medium with Mucolytic | DNA-sorb-AM AmpliSens®MAGNO-sorb-URO | 1x10 ³ |
| | Transport Medium TM-EDEM | EDEM | |
| Discharge from the rectal mucosa | Transport Medium with Mucolytic | DNA-sorb-AM AmpliSens®MAGNO-sorb-URO | 1x10 ³ |
| | | AmpliSens®MAGNO-sorb-URO | |
| Discharge from erosive-ulcerative elements of the skin | Transport Medium with Mucolytic | DNA-sorb-AM AmpliSens®MAGNO-sorb-URO | 1x10 ³ |
| | Transport Medium TM-EDEM | EDEM | |
| Prostate gland secretion | Transport Medium with Mucolytic | DNA-sorb-AM AmpliSens®MAGNO-sorb-URO | 1x10 ³ |
| | | AmpliSens®MAGNO-sorb-URO | |
| Conjunctival discharge | Transport Medium with Mucolytic | DNA-sorb-AM AmpliSens®MAGNO-sorb-URO | 1x10 ³ |
| | Transport Medium TM-EDEM | EDEM | |
| Urine ⁴ | — | DNA-sorb-AM AmpliSens®MAGNO-sorb-URO | 2x10 ³ |
| | | EDEM | 1x10 ⁴ |
| Whole venous blood | — | DNA-sorb-B MAGNO-sorb | 5x10 ³ |
| | | DNA-sorb-B MAGNO-sorb | 5x10 ³ |

⁴ Urine samples are to be pretreated.

NOTE: The concentration is indicated in 1 ml of whole venous blood or cerebrospinal liquid or in terms of 1 ml of a transport medium containing a swab/scrape.

The claimed limit of detection is achieved while respecting the rules specified in the section "Sampling and Handling".

13.2. Analytical specificity

The analytical specificity of **AmpliSens® HSV I, II-FRT** PCR kit is ensured by the selection of specific primers and probes as well as stringent reaction conditions. The primers and probes have been checked for possible homologies to all sequences published in gene banks by sequence comparison analysis.

The PCR kit detects *Simplexvirus humanalpha* 1,2 (HSV I, II) DNA fragments. The analytical specificity was confirmed on the investigating of DNA of following microorganism/strains and human genomic DNA:

- strains from ATCC® (American Type Culture Collection, USA): *Simplexvirus humanalpha* 1,2 (HSV I and II types) (ATCC-VR-539DQ и ATCC-VR-540DQ) in concentration no less than 1×10^3 GE/ml;
- strains from ATCC® (American Type Culture Collection, USA): *Candida albicans* (ATCC® 14053™); *Candida krusei* (ATCC® 14243™); *Escherichia coli* (ATCC® 25922™ и ATCC® 35218™); *Gardnerella vaginalis* (ATCC® 14018™); *Mycoplasma genitalium* (ATCC-49123); *Neisseria gonorrhoeae* (ATCC® 49926™); *Streptococcus agalactiae* (ATCC® 12386™ и ATCC® 13813™); *Staphylococcus aureus* (ATCC® 29213™ и ATCC® 25923™); *Streptococcus pyogenes* (ATCC® 19615™); *Trichomonas vaginalis* (ATCC-50148) in concentration no less than 1×10^3 GE/ml;
- clinical samples (the species identification was confirmed by direct sequencing of nucleotide sequences): *CMV*; *HPV*; *Candida glabrata*; *Chlamydia trachomatis*; *Lactobacillus* spp.; *Mycoplasma hominis*; *Neisseria flava*; *Neisseria mucosa*; *Neisseria sicca*; *Neisseria subflava*; *Toxoplasma gondii*; *Treponema pallidum*; *Ureaplasma urealyticum*; *Ureaplasma parvum* in concentration no less than 1×10^4 and no more than 1×10^8 GE/ml;
- human DNA in concentration of 0.2 mg/ml.

The nonspecific responses were absent while testing DNA samples of the above-mentioned microorganisms and human DNA.

The information about interfering substances is specified in the *Interfering substances and limitations of using test material samples*.

13.3. Reproducibility and repeatability

Repeatability and reproducibility were determined by testing of positive and negative model samples. Positive samples were quality control samples (QCS) containing HSV I, II DNA in concentration of 1×10^4 GE/ml. Negative control (C-) reagent was used as a negative sample.

Repeatability conditions included testing in the same laboratory, by the same operator, using the same equipment within a short period of time. Reproducibility conditions included testing different lots of PCR kit in different laboratories, by different operators, on different days, using different equipment. The results are presented in Table 8.

Table 8

| Sample type | Repeatability | | Reproducibility | |
|-------------|-------------------|-------------------------|-------------------|-------------------------|
| | Number of samples | Agreement of results, % | Number of samples | Agreement of results, % |
| Positive | 10 | 100 | 40 | 100 |
| Negative | 10 | 100 | 40 | 100 |

14. REFERENCES

1. 2024 European guidelines for the management of genital herpes / R. Patel, B. Moran, E. Clarke [et al.] // Journal of the European Academy of Dermatology and Venereology. – 2025. – Vol. 39. – №. 4.
2. Sexually transmitted infections treatment guidelines, 2021 / K.A. Workowski, L.H. Bachmann, P.A. Chan [et al.] // MMWR. Recommendations and Reports. – 2021. – Vol. 70.
3. Rapid and direct detection of herpes simplex virus type 1 and type 2 by Real-Time PCR method in clinical specimens / M. Rezanezhadi, F. Azadi, S. Marhamati, H.R. Joshaghani. // Medical Laboratory Journal. – 2023. – Vol. 17. – №. 6. – P. 8-9.

15. QUALITY CONTROL

In accordance with Federal Budget Institute of Science "Central Research Institute for Epidemiology" ISO 13485-Certified Quality Management System, each lot of **AmpliSens® HSV I, II-FRT** PCR kit has been tested against predetermined specifications to ensure consistent product quality.

List of Changes Made in the Instruction Manual

| VER | Location of changes | Essence of changes |
|----------------|-------------------------------|--|
| 18.08.25 HM | Through the text | Corrections according to the template |
| | 1. Intended use | The intended use was specified. The list of biological material was expanded. The subsection <i>Indications and contra-indications for use of the reagent kit</i> was added |
| | 2. Principle of PCR detection | Section was rewritten |
| | 4. Additional requirements | The section was actualized and updated with materials and instruments |
| | 6. Sampling and handling | The information about sampling and handling was expanded. The subsection <i>Interfering substances and limitations of using test material samples</i> was added |
| | 7. Working conditions | Temperature range was changed. Relative humidity was added |
| | 8. Protocol | Working procedure was rewritten |
| | 9. Data Analysis | Information on the correspondence of the amplification product and channels for the fluorophore, the principle of results interpretation for the test samples and controls are presented in tables |
| | 10. Troubleshooting | The section was rewritten |
| | 11. Transportation | Transportation period was changed from 5 to 10 days |
| | 13. Specifications | The list of microorganisms/strains to prove the analytical specificity was expanded. The subsection <i>13.3. Reproducibility and repeatability</i> was added |
| | 14. References | References were renewed |

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